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RESEARCH PAPER

Histopathological investigation and risk factors of Coccidiosis in camels (*Camelus dromedarius*), Algeria

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Abstract

Coccidiosis in dromedaries is an intestinal protozoan infection caused by apicomplexan parasites belonging to the genus *Eimeria*. A prospective examination was conducted at two slaughterhouses in southern Algeria. The primary objective of this investigation was to ascertain the prevalence of intestinal parasites in camels, elucidate the associated microscopic lesions, and identify the risk factors contributing to this infestation. In pursuit of this, we procured four segments of the intestines from 31 dromedarieas that appeared healthy and displayed no symptoms. Subsequently, these samples were collected, preserved, subjected to routine processing, and subsequently stained with haematoxylin and eosin (H&E). Intestinal parasitic infection showed an incidence rate of 45.16% (14 out of 31). Specifically, two types of parasites were discerned in the intestinal specimens through microscopic examination, namely *Eimeria* (41.93%; 13 out of 31) (p-value = 0.046) and *Taenia* (3.22%; 1 out of 31) (p = 0.001). *Eimeria cameli* was observed in the ceacum (41.93%; 13 out of 31), jejunum (12.90%; 4 out of 31), and in one instance in the duodenum (3.22%; 1 out of 31). Furthermore, numerous development stages of coccidia were identified, including gamonts, schizonts and oocysts. Deep microscopic lesions attributed to *Eimeria cameli* were detected, such as enteritis, eosinophilic infiltration and inflammation. In addition, associated risk factors were identified. This study has furnished valuable insights into parasitic infestations affecting dromedaries, particularly *Eimeria cameli*. The molecular studies are needed to delineate the diverse variations within *Eimeria* strains. Effective parasite control strategies specific to dromedary camels need to be developed.

Keywords

Algeria, camels, coccidiosis, gamonts, histopathological, parasite

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Introduction

Camels (*Camelus dromedarius*) are herbivorous mammals that inhabit the geographical regions of the Middle East and Africa (Amanat et al. 2019). They hold significant importance in the lives of humans, especially in arid areas, due to their multiple roles and their remarkable ability to adapt to challenging conditions (Faye et al. 2014). Their resistance to harsh environmental conditions, such as droughts and high temperatures, makes them extremely valuable. Camels also serve as a vital source of sustenance, supplying milk and meat (Durcic et al. 2020). In Algeria, there is a recorded population of 354,465 camels (Bouasla et al. 2023). While camels may not be the primary domesticated animal, they play a crucial role in providing nutrition for nomadic and urban dwellers, as well as completing various other functions (Saidi et al. 2022). However, this livestock is susceptible to several debilitating diseases (Bennoune et al. 2013; Zait 2016) and gastrointestinal parasite infestations are a common problem, leading to reduce productivity (Mahmuda et al. 2014). One such parasitic ailment is coccidiosis, which holds significant importance in camel health (Dubey et al. 2018). This disease is caused by various species of *Eimeria* spp., belonging to the Phylum Apicomplexa (Utebaeva et al. 2021). *Eimeria* parasites are among the most prevalent pathogens affecting the intestinal tract of many animals. They invade and harm the intestinal epithelium, resulting in severe damage and economic losses (Mehlhorn 2014). Camels, in particular, can be infected by six species of *Eimeria* spp., including *Eimeria bactriani*, *Eimeria cameli* (Kawasmeh and Elbihari 1983). *Eimeria dromedarii*, *Eimeria pellerdy* (Prasad 1960), *Eimeria rajastani* (Dubey and Pande 1963), and *Isospora orlovi* (Tsygankov 1950). In Algeria, there is a scarcity of data regarding gastrointestinal parasitic infections in dromedaries (Baroudi et al. 2018; Laatamna et al. 2018). Only a limited number of studies have investigated these illnesses (Saidi et al.2022). Notably, *E. cameli* and *E. dromedarii* are frequently associated with disease conditions (Djerbouh et al.2018). Due to the limited information available on digestive parasitic infections in dromedaries in Algeria and the objective of shedding light on intestinal coccidiosis, we deemed it highly valuable to initiate a study aimed at detecting digestive parasites, specifically those affecting the intestines of camels (*Camelus dromedarius*) in arid regions, through microscopic and histopathological examinations.

Material and methods

Ethics statement

All animal studies were conducted with the utmost regard for animal welfare, and all animal rights issues were appropriately observed. No animal suffered during the course of this research. All experiments were carried out according to the guidelines of the Institutional Animal Care Committee of the Algerian Higher Education and Scientific Research (Agreement Number 45/DGLPAG/DVA.SDA.14)

Study sites

A cross-sectional study was conducted between november and december 2022, focusing on camels from two slaughterhouses situated in two southern provinces of Algeria. The first slaughterhouse is the Royal abattoir located in the El-Oued province in the northeastern part of the Algerian Sahara, approximately 512 kilometers from the capital city of Algiers. The second slaughterhouse is the communal abattoir of Tamanrasset, located in the far south of Algeria, around 1900 kilometers from the capital city of Algiers.

Samples collections

We obtained 31 samples from clinically healthy dromedaries designed for meat consumption of both genders and various age groups (estimated based on the camel dental equation). These specimens included different segments of the intestinal tract, such as the duodenum, jejunum, ileum, and ceacum, which were collected in *post-mortem* examinations (Fig. 1).

In our study, we used routine methods involving visual inspection, palpation, and incision, to detect any anomalies, according to manual on meat inspection for

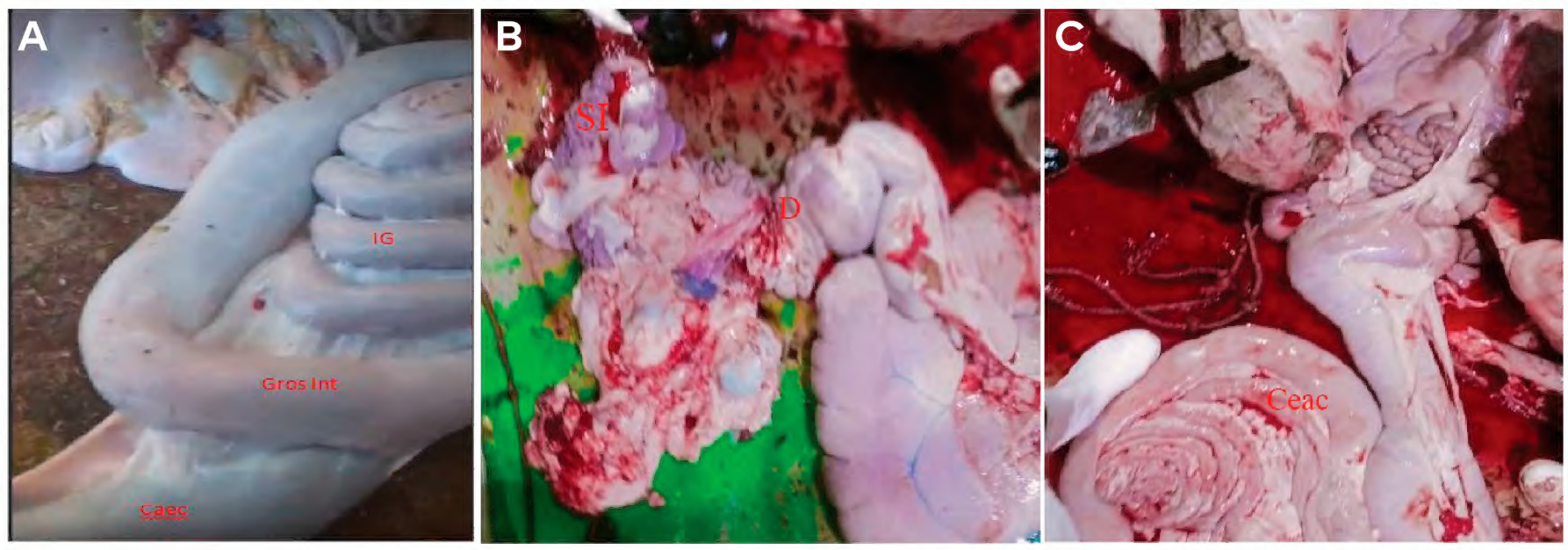

Figure 1. Different intestinal sampled. **A** IG: Small intestine, Caecum; **B** D: duodenum; SI: Small Intestine; **C** Ceac: ceacum.

developing countries (Herenda et al.2000). The samples from the intestine were promptly preserved in 10% neutral buffered formalin without scraping the mucosa. Then, transported to the anatomical pathology laboratory at the Veterinary Institute of Tiaret University in Algeria for subsequent histopathological analysis.

Histopathological analysis

The histopathological analysis was conducted at the Veterinary Sciences Institute's anatomical pathology laboratory at Tiaret University, Algeria. To perform this analysis, tissue samples were first embedded in paraffin and then sliced into sections with a thickness of 5 micrometers using a rotary microtome. These sections were subsequently deparaffinized using xylene and rehydrated through a series of ethanol grades. Following this preparation, they were stained using hematoxylin and eosin (H&E), as described by Luna (1968) The histological sections of the intestine were examined using a Zeiss optical microscope captured using a camera. This histopathological examination aimed to evaluate the morphological characteristics of the intestinal fragments and identify the presence of intestinal parasites, with a particular focus on *Eimeria* in the dromedaries under investigation.

Statistical analysis

Collected data were entered, coded and stored in a Microsoft Excel spread sheet for Windows 2007. A descriptive analysis of the data was carried out. A correlation analysis for the numerical variables was carried out to measure the strength and direction of the relationship between them. The correlation matrix provides a measure of the linear relationship between pairs of numerical variables. The correlation coefficient ranges from -1 to 1, where -1 indicates a perfect negative linear relationship, 1 indicates a perfect positive linear relationship, and 0 indicates no linear relationship and the correlation was presented in the form of a heat map. The Shapiro-Wilk normality test was used for the numerical columns in the dataset for this study.

Results

In this study, the overall prevalence of intestinal parasites in camels was 45.16% (14 out of 31) as shown (Table 1). Through microscopic examination, two types of parasites were identified in the intestinal samples: *Eimeria cameli* (41.93% – 13 out of 31) ($p = 0.046$) which is characterized by the largest oocysts. However, the presence of giant schizonts of various stages "microgamonts, macrogametocytes and oocysts" Fig. 2 present in the lamina propria of the intestinal mucosa made it possible to identify *E.cameli* and we have also isolated Taenia (3.22% – 1 out of 31), ($p = 0.001$).

Table 1. Prevalence rate of intestinal parasites in camels.

Number of camels examined	Number of infected camels	P %
31	14	45.16

P: Prevalence.

The variables different stages of *Eimeria* and *Taenia* appear to follow a normal distribution as their $p > 0.05$. *Eimeria* infestation was most prevalent in the cecum (large intestine) at 41.93% (13 out of 31), followed by the jejunum at 12.90% (4 out of 31), and the duodenum, Ileum at 3.22% (1 out of 31) respectively (Table 2).

Table 2. *Eimeria* infection rates in the intestinal segments.

Intestinal segment	Number of infected camels	P* %
Duodenum	01	3.22
Jejunum	04	12.90
Ileum	01	3.22
Ceacum	13	41.93

Identification of Coccidiosis

Microscopically, large cyst-like structures and immature oocysts were observed mainly in the jejunal and caecal mucosa. According to Dubey et al. (2018), these developmental, cyst-like forms are giant schizonts (microgametes) of *E. cameli* in Fig. 2A, B. Some contained either delicate granules or small spherules Fig. 2C, D. They tended to be embedded at different depths in the mucosa, but predominantly at the base (Fig. 2E). The earliest macrogamont presented a centrally located nucleus, and a prominent nucleolus Fig. 2F. Macrogamonts were large with a central nucleus and peripheral plastic granules (Fig. 2G). As a consequence of the presence of giant schizonts, microgamonts, macrogametocytes, and oocysts of *E. cameli*, the villi, and crypts were distended and disorganized. A moderate to severe mononuclear and eosinophilic infiltration was evident in the lamina propria of the intestinal mucosa.

Histopathological finding

In the present study, diffusely and circumferentially, the duodenum shows acute (92.86%) severe (85.71%) enteritis with (42.86%) of severe congestion (Fig. 3E). Erosion of (78.57%) of the villi was observed (Fig. 3A), and the intestinal crypt epithelium was necrotic in (78.57%) of cases (Fig. 3F). Villi fusion was seen in 28.57% of cases, with dilated lacteals in the same percentage (Fig. 3D). Areas of ulceration with loss of mucosal architecture and replacement by eosinophilic cellular, karyorrhectic debris, was with an associated thick exudative layer of fibrin present in (35.71%) of cases (Fig. 3B). The lamina propria displayed significant expansion due to a severe inflammatory infiltrate in (57.14%) of cases, primarily composed of eosinophils (92.86%), as well as lymphocytes, plasma cells, and neutrophils (Fig. 3C). Only one case out of (92.86%) showed the presence of coccidia.

In regards to the **ileum**, lymphocytic enteritis (78.57%) and eosinophilic (57.14%), sub-acute (71.43%) diffuse,

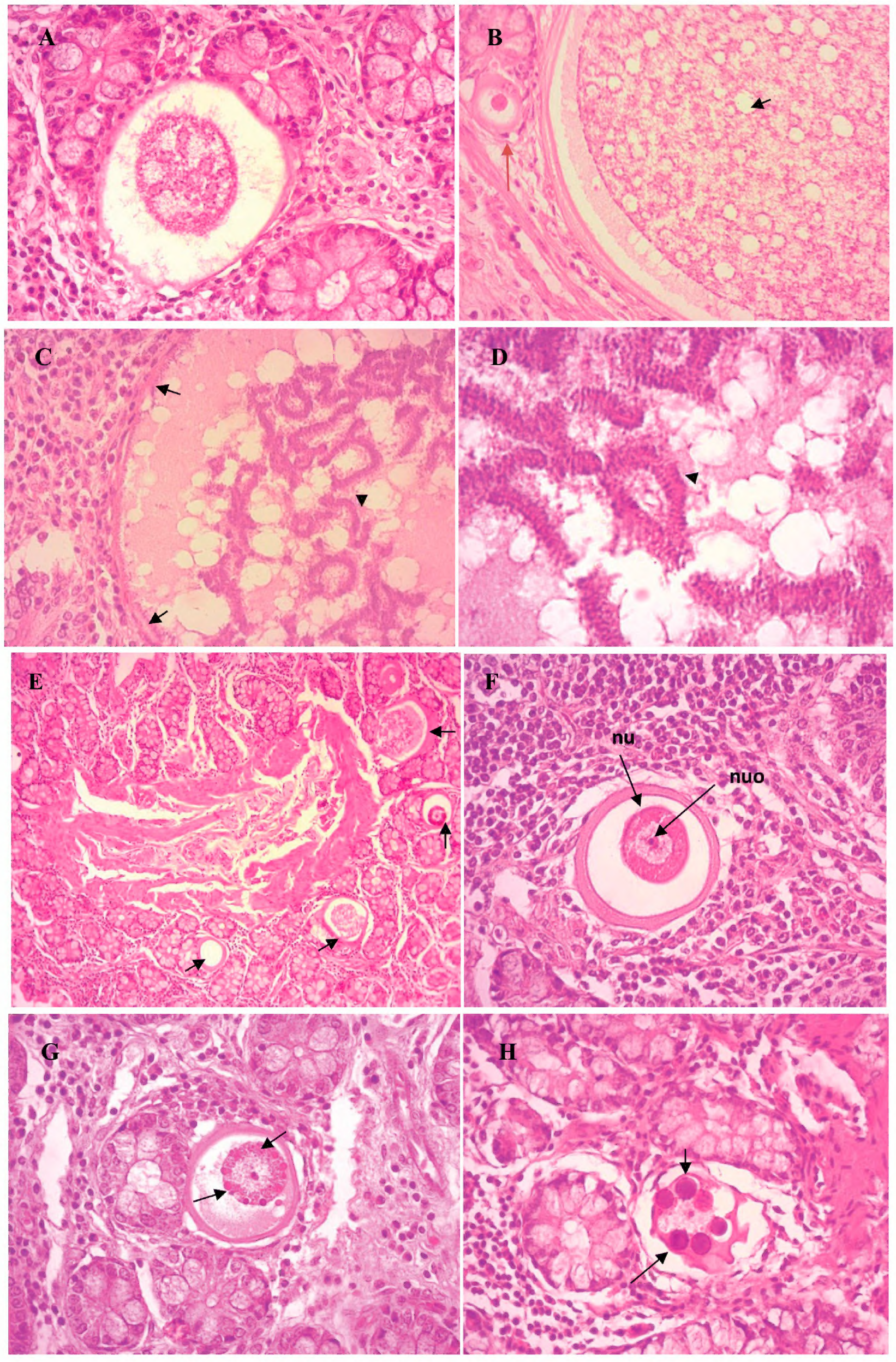

Figure 2. Section of the jejunum, ileum and ceacum of camel showing developing stages of microgamonts of *Eimeria cameli*. **A** Microgamont with several indistinct nuclei (arrow). **B** The crypts of Lieberkuhn are obliterated due to the growth of microgamont, note the numerous nuclei arranged in blastophores (arrowheads). An Earliest microgamont lies below the large schizont (red arrow) ×400. **C, D** Microgametes arranged centrally in rows (arrowheads) and at the periphery (arrow) ×1000. **E** Different stages of *Eimeria cameli* at the base of the mucosa ×100. **F** Macrogamonts in an early stage, the nucleus is prominent, as well as the nucleolus (nuo) ×400. **G** Developing macrogamont with the wall-forming bodies (arrow) ×400. **H** Macrogamont with varying-sized of wall-forming bodies (arrowheads) ×400. H&E stain.

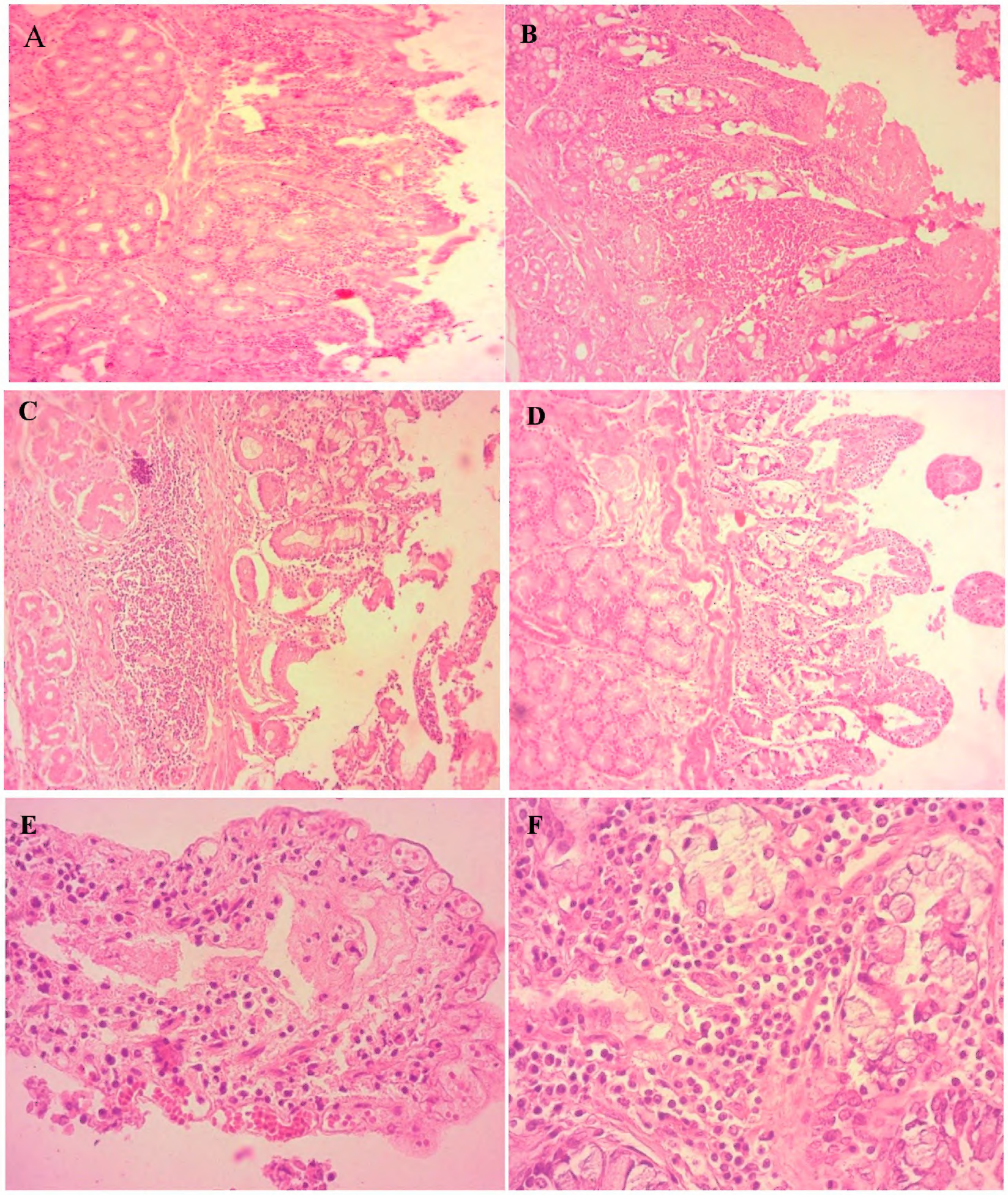

Figure 3. Duodenum **A** acute, severe enteritis ×100; **B** Presence of a fibrino-necrotic exudate at the top of the villi ×100; **C** Necrosis of villi and crypts with expansion of the lamina propria by inflammatory cells ×100; **D** Lacteals within the villi are moderately dilated ×100; **E** Loss of epithelium villi with congestion, hemorrhage and inflammation ×400; **F** Crypt epithelial cells are necrotic. Crypt lumens contain karyorrhectic debris ×400.

moderate, with villus erosion noted in 92.86% of cases (Fig. 4A). The mucosal epithelium was eroded in 92.86% of cases (Fig. 4B), while 64.28% of crypts maintained their normal structure. Various developmental stages of coccidian parasites, such as macrogamonts, microgamonts, and oocysts, were identified in 21.43% of cases (Fig. 4C). Peyer's patches (GALT) in the submucosa of the ileum contained active germinal centers in 28.57% of cases (Fig. 4D), and moderate to severe inflammatory infiltrate consisting of lymphocytes and eosinophils expanded the lamina propria and submucosa in 50% of cases (Fig. 4E, F).

At the level of the **Jejunum**, acute (78.57%), diffuse, severe (71.43%), with marked villus erosion (85.71%) was observed. Additionally, 78.57% of the villi contained various developmental stages of coccidian parasites, including macrogamonts, microgamonts, and oocysts, in varying percentages (Fig. 5C,D) Marked necrosis and loss of crypts were diffusely present (57.14%), and the lamina propria exhibited significant expansion with presence of eosinophils (71.43%), plasma cells (28.57%), and lymphocytes (21.43%).

In the **ceacum**, circumferentially, chronic-active (85.71%), severe (71.43%), eosinophilic (78.57%), diffuse, typhlitis was predominant (Fig. 6A) with important congestion (57.14%), 100% of the villi were eroded, shortened and filled with inflammatory cells mainly eosinophils (Fig. 6B). The lamina propria was markedly

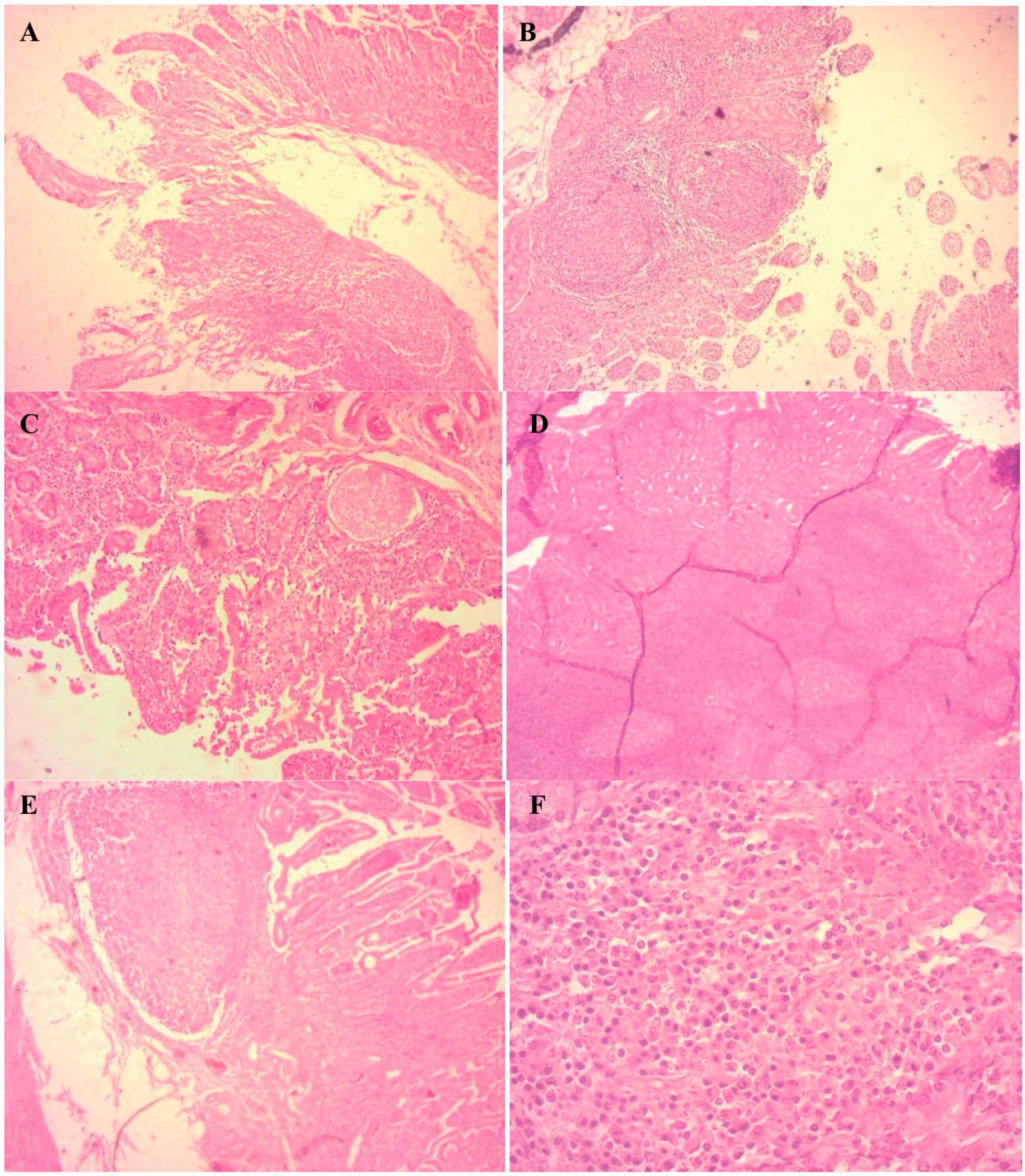

Figure 4. Ileum **A** lymphocytic and eosinophilic enteritis with villus erosion ×40; **B** Complete erosion of villi and crypts ×40; **C** Various developmental stages of coccidian parasites, such as macrogamonts, microgamonts, and oocysts within the crypts ×100; **D** Lymphocyte reaction (germinal centre); **E, F** The lamina propria is infiltrated by inflammatory cells composed of lymphocytes, eosinophils and plasma cells ×100, 400. H&E.

expanded (71.43%) by inflammatory cells, primarily eosinophils (78.57%). Crypts were predominantly necrotic (92.86%) and separated widely by a diffuse eosinophilic infiltrate (Fig. 6C). Abundant coccidian parasites were found within the crypts, with various developmental stages, including macrogamonts, microgamonts, and oocysts, primarily located in the lamina propria to submucosa (Fig. 6D, E). Cryptitis was seen in (35.71%) of the cases (Fig. 6F).

Risk factors

The study identified certain risk factors associated with *Eimeria* infestation in dromedaries, including breed, sex, area, and age, as outlined (Table 3).

The results of the correlation matrix for the numerical variables in this study are presented in the heatmap (Fig. 7). The heatmap illustrates that most of the variables are not strongly correlated with each other, as indicated

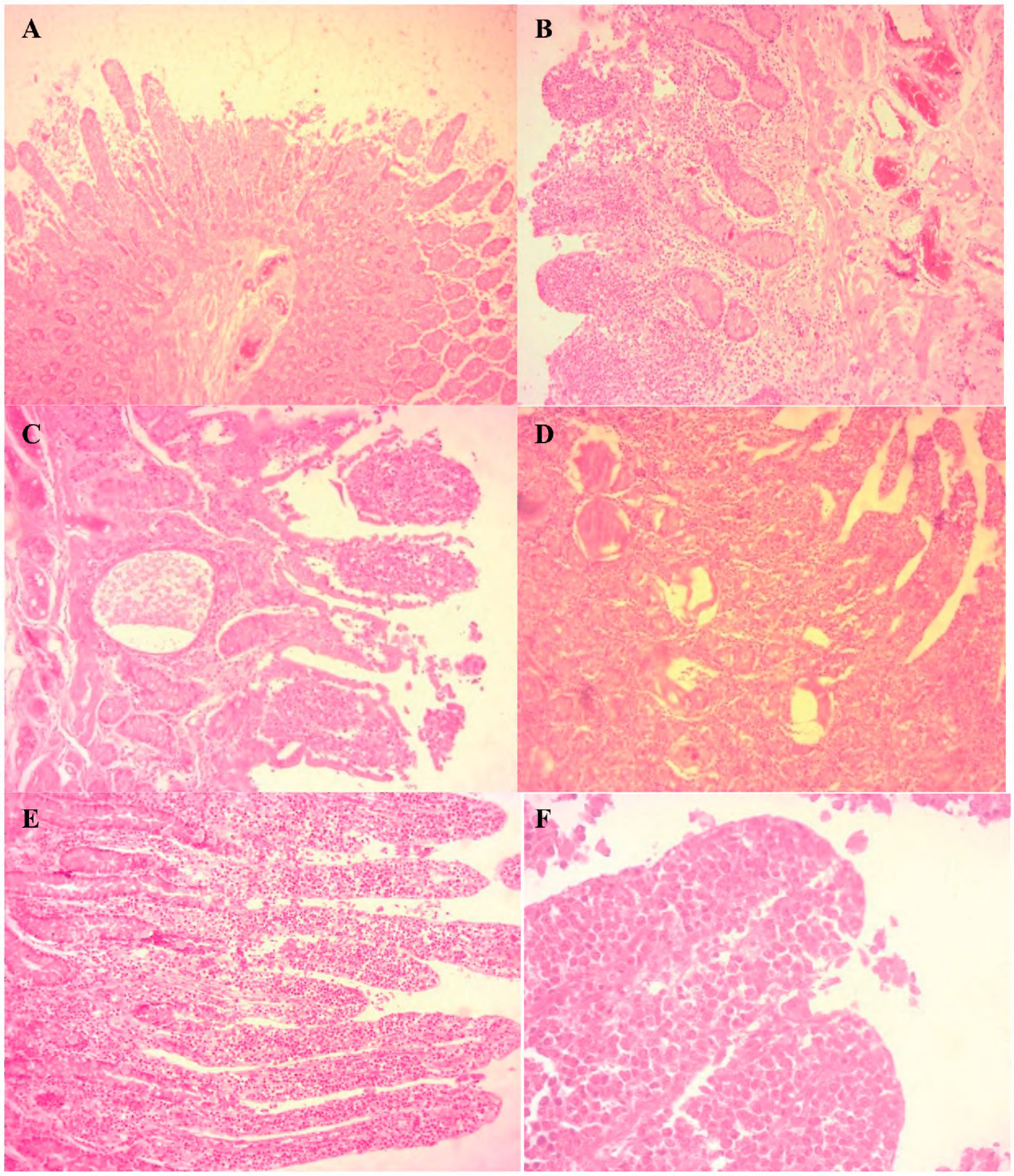

Figure 5. Jejunum. **A, B** Acute, diffuse , severe enteritis with marked villus erosion and severe congestion ×40, ×100; **C, D** Various developmental stages of coccidian parasites, such as macrogamonts, microgamonts, and oocysts within the crypts ×100; **E** Significant eosinophilic infiltration ×100; **F** villus filed with eosinophilic infiltration ×400. H&E.

Table 3. Distribution and risk factors of *Eimeria* camels' infection.

Parameters	IC (%)	NIC (%)
Area		
El-Oued	35.48	64.52
Tamanrasset	6.45	93.55
Breeds		
Sahraoui	35.48	64.52
Targui	6.45	93.55
Sex		
Male	12.90	87.10
Female	29.03	70.97
Age Group		
Young	6.45	93.55
Adult	6.45	93.55
Old	29.03	70.97

IC* Infection coccidiosis; NIC* Non infection coccidiosis.

by the light colors. Additionally, it was found that most of the variables did not follow a normal distribution, as their $p < 0.05$ (Fig. 7).

Discussion

Coccidia, specifically from the genus *Eimeria*, are protozoan parasites known for their high efficiency in residing and multiplying within the intestinal tract, as noted by Hamidinejat (2010). This study presents interesting results regarding parasitic infestations in the intestines of dromedaries in two slaughterhouses located in the southern region of Algeria. Microscopic examination revealed a parasitic infection rate of 45.16% (14 out of 31). Previous research has been

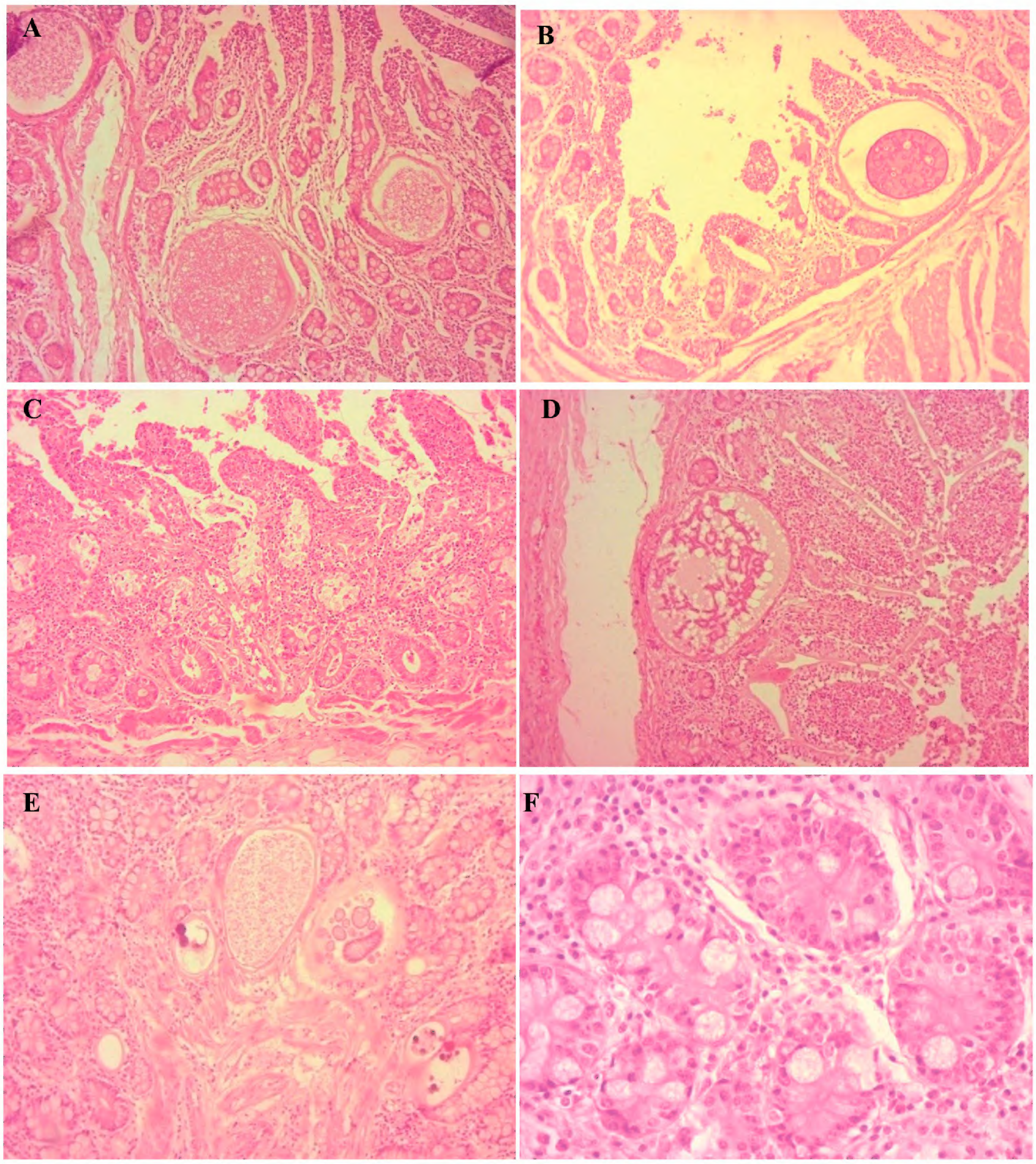

Figure 6. Ceacum **A** chronic-active , severe, eosinophilic , diffuse typhlitis ×100; **B** Villi eroded ,shortened and filled with inflammatory cells mainly eosinophils ×40; **C** Necrosis of crypts ×100; **D, E** Presence of numerous stages of parasites ×100; **F** Cryptitis with numerous intraepithelial and luminal neutrophyls ×400.H&E.

based exclusively on studies of intestinal parasites in faeces such as those reported by Djerbouh et al. (2018), Bouragba et al. (2020) and Saidi et al. (2021). To our knowledge, no studies have been carried out on the histopathological examination on intestinal parasites of dromedaries in Algeria. However, it's important to note that our reported prevalence rates are lower than those documented in Somalia, where Ibrahim et al. reported a prevalence of 50.3% (Ibrahim et al. 2016). These variations in prevalence rates across different regions highlight the diverse nature of parasitic infestations in dromedaries and suggest that factors such as geographical location, environmental conditions, and herd management practices may contribute to differences in parasitic prevalence among camel populations. In our study, two types of parasites were identified, with *Eimeria* being the most prevalent at 41.93% (13 out of 31), and *Taenia* (3.22%; 1 out of 31) This association between helminthic and protozoan infections was reported by Bouragba et al. (2020) and Saidi et al. (2022) in studies of intestinal parasites by coprological examination of dromedary in Algeria.

In contrast, the prevalence of *Eimeria* in this study is higher than that reported by Kawasmeh and Elbihari (1983), who indicated (20%) of *E. cameli* oocysts in intestinal scrapings from slaughtered camels in Saudi Arabia. Whereas, our results are inferior to those reported by Tafti et al. (2002), who recorded that the most important lesions and their frequency of occurrence result from *Eimeria* spp. infections (63%) in a study of histopathological lesions in

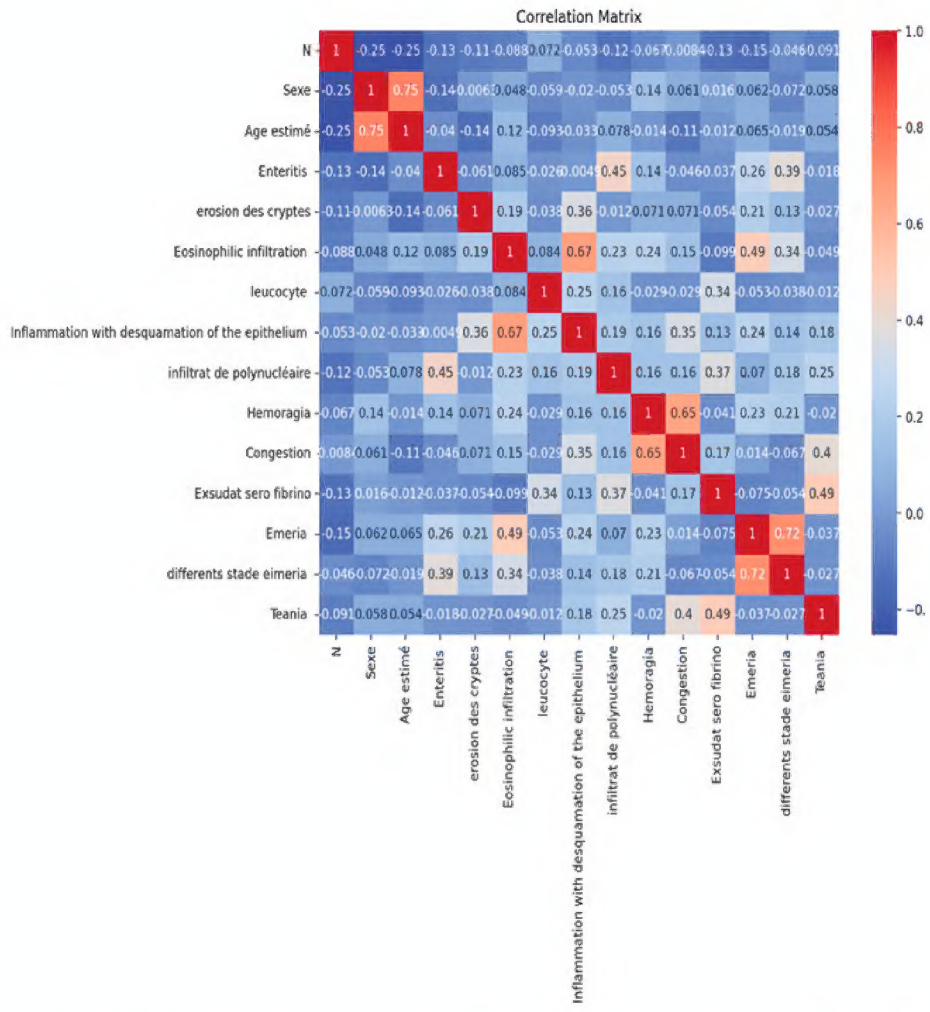

Figure 7. Correlation matrix for the numerical variable.

the small and large intestines of 100 camels slaughtered in Iran. Our study specifically noted that *Eimeria* was more prevalent in the large intestine, we also confirmed the presence of various sexual stages of *Eimeria*, including gamonts, schizonts, and oocysts, in the large intestine (ceacum) of camels. Interestingly, the distribution of *Eimeria* stages in different parts of the intestine varies in the literature. Kinne et al. (2002) found numerous coccidian stages (gamonts and oocysts) in the large intestine, whereas Kinne and Wernery (1997) identified different stages of *Eimeria* in the small intestine. Additionally, Dubey et al. (2018) reported that the developmental stages of *Eimeria* macrogamonts were located in the small intestine (ileum) of camels.

These variations highlight the diversity in the localization of *Eimeria* stages in camel intestines across different studies. We observed various microscopic lesions in the camel intestines, with a notably high rate of inflammation and desquamation of the epithelium in the duodenum (76.92%), followed by the jejunum and ceacum (46.15%). These findings are consistent with the relative numbers of eosinophils present in the intestinal samples. Additionally, we observed gross lesions characterized by enteritis with hemorrhages on the mucosal surface and erosion of the intestinal epithelium, particularly in the crypts. Our observations align with previous studies by Fanatico (2006); Marquardt et al. (2000); Youssef et al. (2015); Sharma et al. (2015) which reported similar histopathological findings in the intestines of birds infected with various *Eimeria* species. We also noted the presence of different stages of *Eimeria* development, marked hemorrhage, and congestion in the large intestine, confirming the results reported by Salem et al. (2022). *Eimeria* parasites are recognized as primary pathogens of the intestinal tract in many animals, causing invasion and damage to the intestinal epithelium, a phenomenon noted by Mehlhorn (2014.). Consistently in agreement with Dubey et al. (2018), *E. cameli* spread throughout all regions from the lamina propria to submucosa, especially in the Lieberkuhn crypts. The same finding was reported by Hussein et al. (1987) in a study carried out in Saudi Arabia. In our study on risk factors for *Eimeria* infection in dromedaries, we found that camels from the El-Oued region had the highest infestation rate at (35.48%), compared to Tamanrasset at (6.45%). Additionally, the Sahraoui breed appeared to be more susceptible to infestation compared to the Targui breed. This difference could potentially be explained by the higher density of the Sahraoui breed in the study area, a pattern also observed by Saidi where Laghouat showed the highest infection rate (45.5%) compared to other sites (Saidi et al. 2022).

Furthermore, Bouasla et al. (2023) reported variations in the rate of parasite infestation in camels across different regions in Algeria, including Ouargla (73.68%), Adrar (18.18%), Ain Salah (10%), and Tindouf (33.33%). These variations can be attributed to differences in agroclimatic conditions, hygiene levels, and animal husbandry practices between regions and even between countries (Allport et al. 2005). Female dromedaries exhibited a higher infection rate of coccidiosis at 29.03% compared to males at 6.45%. This observation aligns with findings from Bekele (2002); Bamaiyi and Kalu (2011); Abdel-Rady (2014), who all reported that females were more susceptible to parasites than males. This increased susceptibility in female camels may be attributed to their physiological characteristics, which can act as stress factors and reduce their immunity to infections, as noted by Wakelin (1984). Furthermore, older dromedaries showed a higher prevalence of intestinal parasites at 29.03%, followed by other age groups, including adults and young animals. This can be explained as Older dromedaries are more susceptible to coccidial infection than other age groups. This may be due to ageing of the intestinal mucosa with loss of elasticity. Also, the intestinal villi are incomplete, which minimizes absorption, and probably the intestinal cells are smaller, so that the immune system is less effective. These dromedaries did not show any clinical signs and are therefore considered healthy carriers. Contrary to, Kaufmann (1996) who reported that young camels were much more susceptible to infection by *Eimeria* spp. than adults. Bouragba et al. (2020) also confirmed that factors such as animal breed, age, and population density can influence the susceptibility to parasitic infections. The correlation matrix revealed a strong positive correlation of 0.747 between sex and estimated age, suggesting that as the sex value increased, the estimated age tended to increase as well. The heatmap displayed relatively strong positive correlations between "Enteritis" and "Crypt erosion," as well as between "Inflammation with desquamation of the epithelium" and "Polynuclear infiltrate." Additionally, "Enteritis" and "different stages of *Eimeria*" had a moderate positive correlation of 0.391, indicating a moderate linear relationship between these two variables. However, "*Eimeria*" and "different stages of *Eimeria*" showed a strong positive correlation of 0.719, indicating a strong linear relationship. Similarly, "Exudate serofibrinous" and "*Taenia*" had a moderate positive cor-

relation of 0.494, suggesting a moderate linear relationship. Most other pairs of variables had correlation coefficients close to 0, indicating weak or nonexistent linear relationships. It's important to note that correlation does not imply causation, and these relationships may be influenced by other unaccounted factors in the dataset. Further research is warranted to enhance our understanding of the impact of these factors on camel health.

Conclusions

This study has provided valuable insights into the prevalence of intestinal parasites, particularly coccidiosis, and the associated risk factors among camels in two regions, El-Oued and Tamanrasset, in Algeria. The findings underscore the significance of camels as reservoirs and healthy carriers for the genus *Eimeria* and highlight the substantial challenge these parasites pose to camel health. As a result, we recommend the establishment of parasite control programs in Algeria, focusing on preventive measures and treatment strategies to mitigate the impact of coccidiosis and other intestinal parasites on camel populations. Additionally, we suggest conducting molecular studies to gain a deeper understanding of the specific parasites and their genetic characteristics, which can aid in developing more effective control and management strategies for camel health in the region. These efforts are essential to ensure the well-being and productivity of camel herds and to safeguard their role in agriculture and livelihoods.

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